COLOR STANDARDS FOR COD LIVER OIL.*

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The present pharmacopœia (1) provides that the color of cod liver oil shall be "pale yellow." Obviously this specification is not sufficiently definite to be of practical value. As noted by Taub (2) the term "pale yellow" "may imply that a colorless oil would not meet the U. S. P. requirements or it may leave the observer in doubt as to whether the oil in question is slightly darker than pale yellow."

After a careful study conducted to obtain satisfactory color standards for cod liver oils, Taub (2) has suggested the use of solutions of cobalt and ferris chlorides as originally proposed by Arny (3). The proposal made by Taub for consideration by the Committee of Revision of the U. S. Pharmacopœia for interim revision of the text of U. S. P. X and for continuation in U. S. P. XI reads as follows:

"The oil, when placed in a 4-ounce, tall, cylindrical, standard oil-sample bottle, and viewed transversely, shall not be more highly colored than a solution placed in a similar bottle and containing 3.6 cc. $M/4$ $CoCl_2 6H_2O$, 48.4 cc. $M/6$ $FeCl_3 . 6H_2O$ and 68 cc. of distilled water. (Or if Standard No. 2 is adopted, these figures would be 11 cc. $M/4$ $CoCl_2 6H_2O$, 76 cc. $M/6$ $FeCl_3 \cdot 6H_2O$ and 33 cc. of distilled water.)

"It would also be necessary to provide for two test solutions: $M/4$ $CoCl_2 6H_2O$ contains 59.4965 Gm. of this salt per liter of 1 per cent HCl. $M/6$ $FeCl_3 . 6H_2O$ contains 45.054 Gm. of this salt per liter of 1 per cent HCl. Provision should be made for standardizing these solutions volumetrically, as described in a previous paper."

Obviously this specification is a decided improvement over the present provision relative to "pale yellow" oil but it is subject to some criticism. It is difficult to accurately estimate the intensity of color by viewing transversely cylindrical bottles of oil. The layer of oil to be viewed is over an inch thick at the center and rapidly decreases to no thickness at the sides; the glass sides of four-ounce, tall, cylindrical, standard oil-sample bottles are not uniform when viewed transversely; and no provision is made for a uniform source of light. It was with a desire to improve upon these features of the proposed U. S. P. color standards for cod liver oil that this study was undertaken.

In comparing the color of a cod liver oil and the standard inorganic salt solution it is essential to view them through layers of equal depth and this was accomplished by using 50-cc. Nessler Tubes (A. P. H. A. type). These, made of clear, colorless glass with polished bottoms, may be purchased of chemical supply houses in Matched Sets of six or twelve tubes. Thus the operator is assured of columns of oil and standard of the same surface area and of the same depth. Moreover, since the columns of oil and standard are eight and one-half inches deep a far more accurate comparison can be obtained than is possible by viewing layers of oil and standard which vary from about one and one-third inches to 0 inches in depth. Moreover in comparing the colors of columns of oil and standard by viewing them vertically in Nessler tubes one looks directly at the oil and standard instead of viewing variable depths of oil and standard through convex glasses.

Since the composition and intensity of daylight varies with the location of different laboratories; with the season of the year; from day to day; and even

* Presented before the Division of Medicinal Chemistry, American Chemical Society, Chicago meeting.

from hour to hour of the same day it seemed highly desirable to provide a source of light which could be conveniently duplicated in other laboratories. Taub (2) has very wisely pointed out that the method for testing the color of cod liver oil should be simple and inexpensive. Accordingly the apparatus devised for this study requires a minimum of materials and is easy of construction. If the services of a cabinet maker are available and the finished apparatus is carefully stained, a handsome piece of laboratory equipment can be produced. On the other hand any laboratory assistant who is at all adept with carpenters' tools can make the apparatus satisfactory for practical needs. The external appearance of the apparatus is illustrated in Fig. 1 and the details of its construction are shown in Fig. 2. One

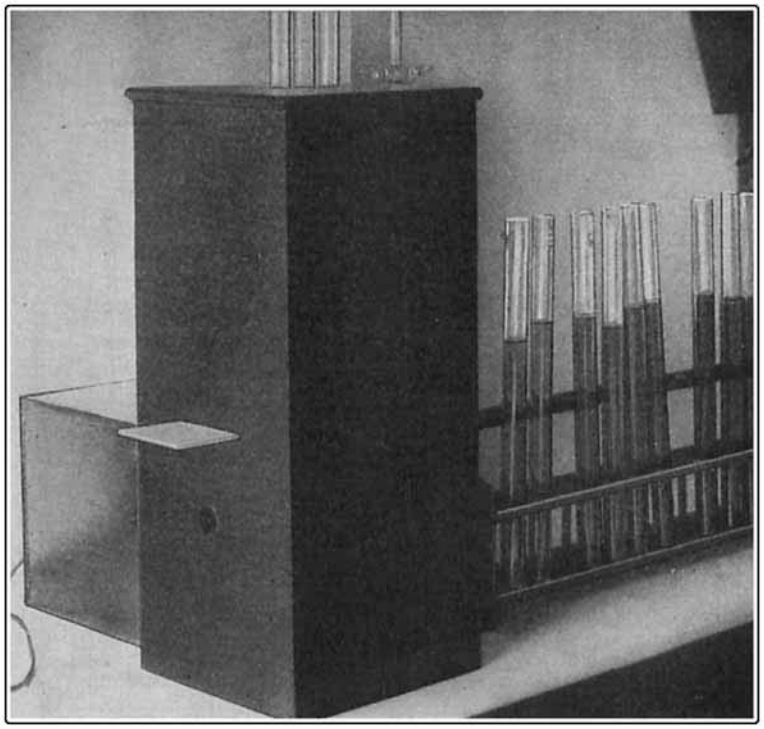

Fig. 1.

feature not shown in Fig. 2 should be provided. Holes or slits should be made in the bottom and top or top of the sides of the lamp section to provide sufficient ventilation to prevent scorching the paint on the interior walls, for this would decrease the reflection of light to the under side of the ground glass upon which the Nessler tubes stand. In this connection it may be noted that the lamp, 100 Watt, blue, daylight Mazda, which is to be used as a source of light should be so placed that reflected light is obtained. If it is placed directly beneath the Nessler tubes unsatisfactory lighting results. As will be noted in Figs. 1 and 2 a tube of oil and of standard, or two tubes of standards with a tube of oil between are placed in the apparatus vertically so that the columns of oil and standard can be compared by viewing them vertically.

Coincident with tests of the color of an extensive series of cod liver oils a study was made of the permanence of the color of the proposed standard solution. A quantity of the standard solution was placed in four-ounce, tall, cylindrical, oil-sample bottles since it was felt that thus the surface area exposed to light action would be equal to or greater than that of solutions stored in reagent bottles under average laboratory conditions. One of the bottles was placed in a wood cupboard from which practically all light was excluded. The second was placed on a laboratory reagent shelf. The third was placed on the roof and thus exposed to three

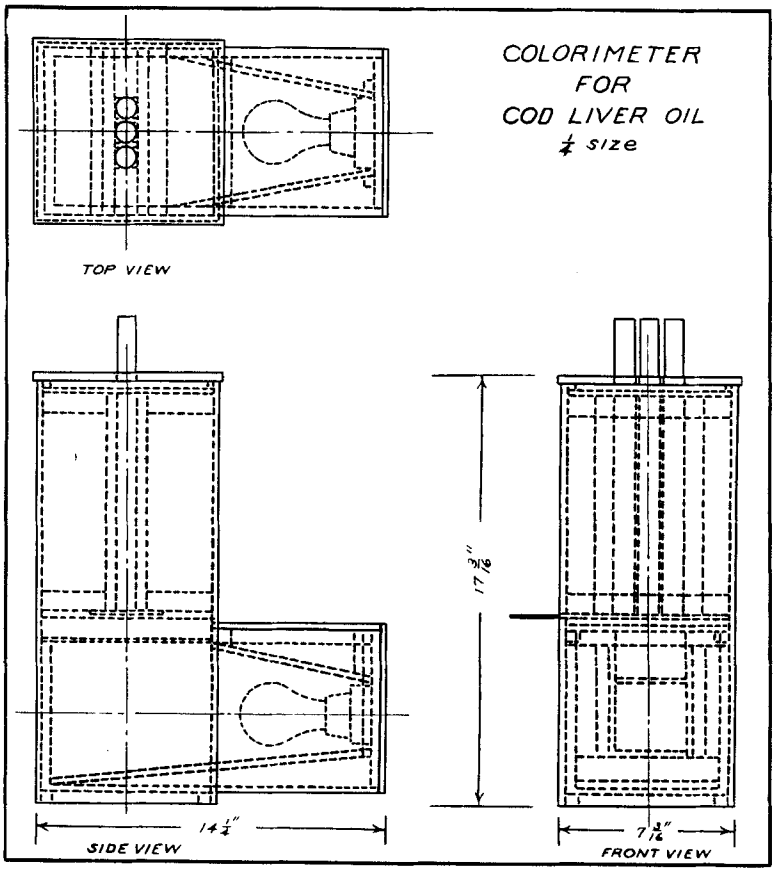

FIG. 2

weeks of late May sunshine and the fourth bottle was subjected to Fade-ometer tests (4). The Atlas Electric Device Co. instrument that was used in these tests was provided with violet carbon arcs which had an electricity consumption of 13 amperes at 220 volts. It was claimed that one-quarter hour of the Fade-ometer light was equivalent to one hour of maximum intensity, noonday, June sunlight at 41° 50′ N. latitude. The standard solution was exposed to this light for 65 hours which would be equivalent to 260 hours of noonday, June sunlight at the latitude noted. Tests, made in the apparatus described above, of the color of the four samples five weeks after they were prepared revealed no difference in the color of

samples Nos. 1, 2 and 3. The color of sample No. 4 was noticeably though not seriously darker than sample No. 1 which was used as a control. However, since the heat and light to which sample No. 4 was subjected was so much more intense than that to which reagents are subjected it is assumed that the color of the standard solution is sufficiently "permanent" to remain constant during the period that standard solutions are ordinarily used for accurate quantitative analyses.

While the primary purpose of determining the color of a series of cod liver oils to be discussed later was to ascertain what proportion of average cod liver oils complied with the proposed color standard it seemed highly desirable to also determine to what extent the oils deviated from the standard. To provide such data additional solutions were prepared. Four of these which retained the shade but not the intensity of the color of the standard (U. S. P. No. 1) were prepared by diluting the standard with varying amounts of distilled water. These solutions numbered in sequence in order of increasing intensity of color constituted the first portion of the color scale used in this study. As will be noted on inspection of the formulas of the proposed U. S. P. standards, "Standard No. 2" which is suggested as the maximum color for medicinal oil is more intense and of a darker shade than "Standard No. 1" which is also suggested as the maximum color for medicinal cod liver oil. Accordingly this difference was divided into four steps of increasing intensity and redness by preparing three solutions containing the requisite amounts of cobalt and iron chlorides. Hence, the color scale employed in this study consisted of nine solutions of cobalt and iron chlorides of which No. 5 and No. 9 were the proposed U. S. P. standard solutions No. 1 and No. 2, respectively. The composition of the nine solutions computed on a liter basis are reported in Table 1.

The series of over one hundred cod liver oils that have been compared with this color scale may be conveniently classified as crude oils, medicinal oils of American and European origin, old medicinal oils and cod liver pressings or cod liver stearin. Since the latter is a somewhat variable product and is little used for human consumption the results obtained in a comparison of these with the color scale will not be discussed here. Inasmuch as the free fatty acid content of cod liver oil is sometimes considered as indicating the age of an oil a free fatty acid determination was made for each of the oils studied.

Since crude cod liver oil is the oil as it is originally obtained from cod livers and since crude cod liver oil is the source of all medicinal cod liver oil the results obtained for the crude oils will receive first consideration in this discussion. These oils, which were principally of American origin, were produced at points along the Atlantic Coast from Cape Cod to Northern Newfoundland. The values assigned to the color of these oils and their free fatty acid content are reported in Table 2. In the absence of any data to the contrary it has been assumed that these oils have not received any alkali treatment and that the acid values represent the condition of the oils as they were extracted from the livers. An inspection of Table II reveals that only 16 of the 54 oils met the proposed U. S. P. Color Standard No. 1 for medicinal cod liver oil. Hence, 38 of this series of oils would have been denied entry to the United States had this proposed color standard been in force. Of these 38 oils six were without doubt too dark for use in the manufacture of medicinal oil. However, a question may properly be raised concerning the desirability of excluding

from the country those crude oils which are not greatly darker than the proposed U. S. P. Standard No. 1. Three of the oils under discussion, Nos. 1310, 1386 and 1440, were taken from large lots of crude oil which were subsequently chilled and pressed in the routine manufacture of cod liver oil. The resulting medicinal oils Nos. 1316, 1409 and 1452, respectively, when sampled and compared with the color standards were found to be significantly lighter in color. If these results can be considered as typical of crude oils then 32 of the oils reported in Table 2 as not meeting the proposed U. S. P. Standard No. 1 would comply with the specifications when chilled and pressed.

TABLE I

COLOR OF COD LIVER OIL

FORMULAE FOR STANDARDS

STANDARD NO.	SOLUTION A * (C.C PER LITER)	SOLUTION B X (C.C. PER LITER)	DISTILLED WATER (C.C. PER LITER)
1	15.00	201.67	783.33
2	18.75	252.08	729.17
3	22.50	302.50	675.00
4	26.25	352.92	620.83
5	30.00	403.33	566.67
6	45.42	536.00	418.58
7	60.83	618.75	320.42
8	76.25	651.67	272.08
9	91.67	633.33	275.00

* SOLUTION A — 59.4965 GMS $CoCl_2 \cdot 6H_2O$ PER LITER OF 1% HCL

X SOLUTION B — 45.0540 GMS $FeCl_3 \cdot 6H_2O$ PER LITER OF 1% HCL

On referring to Table II it will be noted that the free fatty acid content of the crude oils studied varied from 0.144% for oil No. 1466 to 1.565% for oil No. 1282 It will also be noted that while the U. S. P. X permits a maximum of 1.41% free fatty acid only one of the crude oils, No. 1282, exceeded this value and with the exception of four other oils the fatty acid content of the 54 crude oils is less than two-thirds of the free fatty acid content permitted. In fact many of the oils had a free fatty acid value of only one-fourth or one-third that permitted by the U. S. P. The darkest of the 54 crude oils studied, Nos. 1282, 1396, 1278, 1279 and 1280, have the highest free fatty acid values, namely, 1.565%, 1.033%, 1.022%, 1.075% and 1.053%, respectively. On the other hand the free fatty acid values, 0.463%, 0.428%, 0.339%, 0.505%, 0.927% and 0.804%, which are for the lightest colored oils Nos. 1437, 1318A, 1371, 1405, 1388 and 1447, respectively, were far from the lowest free fatty acid values obtained. Moreover, the free fatty acid values of 0.144%, 0.264%, 0.296%, 0.304%, 0.331%, 0.332%, 0.348%, 0.355% and 0.356% for samples Nos. 1466, 1365, 1361, 1384, 1360, 1385, 1296, 1379 and 1398 are for cod liver oils which are darker than the proposed U. S. P. Standard No. 1. A careful comparison of the free fatty acid content of the oils with the color values assigned to them will show that while there is a tendency for the darker oils to have high acid values and the lighter oils to have decidedly lower free fatty acid values there is no close correlation between the fatty acid value and the color of cod liver oil.

The results obtained for medicinal oils of American origin appear in Table 3. As will be noted on referring to color values only two oils failed to comply with the proposed U. S. P. Standard No. 1 and those were only slightly darker than the

standard. From the results obtained with this limited series of American oils it appears that these oils are of quite satisfactory color.

TABLE 2

COLOR OF COD LIVER OIL

LAB. NO.	CRUDE (AMERICAN)	F.F.A. %	COLOR SCALE 1 2 3 4 5 6 7 8 9
1282	"	1.565	
1405	"	0.505	
1388	"	0.927	
1396	"	1.033	
1445	"	0.905	
1447	"	0.804	
1278	"	1.022	
1279	"	1.075	
1280	"	1.053	
1317	"	0.445	
1336	"	0.382	
1382	"	0.484	
1387	"	0.792	
1389	"	0.710	
1446	"	0.347	
1448	"	0.537	
1475	"	0.553	
1476	"	0.501	
1466	"	0.144	
1320	"	0.587	
1335	"	0.536	
1398	"	0.356	
1438	"	0.620	
1480	"	0.444	
1365	"	0.264	
1366	"	0.499	
1362	"	0.598	
1296	"	0.348	
1297	"	0.459	
1433	"	0.674	
1477	"	0.686	
1364	"	0.483	
1360	"	0.331	
1379	"	0.355	
1385	"	0.392	
1361	"	0.296	
1381	"	0.464	
1384	"	0.304	
1363	"	0.364	
1380	"	0.474	
1298	"	0.441	
1318A	"	0.428	
1367	"	0.357	
1368	"	0.359	
1371	"	0.339	
1400	"	0.498	
1401	"	0.365	
1402	"	0.546	
1437	"	0.463	
1474	"	0.773	
1479	"	0.453	
1310	"	0.520	
1386	"	0.558	
1440	"	0.677	

Thirteen medicinal oils of European origin were compared with the color scale and the results are summarized in Table 4. Five of the oils were very light colored but five others or 38% of the group did not comply with the suggested U. S. P. Color Standard No. 1. Of these two were not greatly darker than the standard. However, if the oils under consideration are typical of European oils

then the adoption of the proposed U. S. P. Standard No. 1 would prevent entry into the United States of about one-third of European medicinal oils.

In considering the color of cod liver oil it seemed pertinent to determine the influence of storage upon color. Under normal storage conditions care is taken to

TABLE 3

COLOR OF COD LIVER OIL

LAB. NO.	MEDICINAL (AMERICAN)	F.F.A. %	COLOR SCALE 1 2 3 4 5 6 7 8 9
829	"	0.561	
1247	"	0.684	
1271	"	0.653	
1288	"	1.050	
1316	"	0.519	
1342	"	0.477	
1376	"	0.442	
1408	"	0.572	
1409	"	0.590	
1411	"	0.576	
1423	"	0.566	
1452	"	0.660	

TABLE 4

COLOR OF COD LIVER OIL

LAB. NO.	MEDICINAL (EUROPEAN)	F.F.A. %	COLOR SCALE 1 2 3 4 5 6 7 8 9
1338	"	0.674	
1339	"	0.499	
1340	"	0.450	
1341	"	0.541	
1343	"	1.050	
1344	"	0.487	
1345	"	0.425	
1350	"	0.490	
1353	"	0.273	
1431	"	0.788	
1442	"	0.392	
1449	"	0.616	
1467	"	0.593	

TABLE 5

COLOR OF COD LIVER OIL

LAB. NO.	MEDICINAL OLD OILS (EUROPEAN)	F.F.A. %	COLOR SCALE 1 2 3 4 5 6 7 8 9
1126	"	0.996	
1459	"	0.936	
1460	"	0.924	
1461	"	1.168	
1462	"	0.868	
1463	"	1.092	
1464	"	1.238	
1465	"	0.928	

protect oil from the air and thus reduce possible atmospheric oxidation to a minimum but for the purpose of this test it seemed desirable to determine the colors of oils that had been subjected to rigorous storage conditions. Accordingly a series of European oils that had been stored under laboratory conditions in partially filled bottles for one to six years were tested. As will be noted on referring to Table 5

five or approximately 60% were found to be darker than the suggested U. S. P. Standard No. 1 but it should be noted that four of these are only slightly darker than the standard. In general these oils are very little darker than the fresh European oils discussed in Table 4. On the other hand the acid values are much higher, in fact in some instances the reported acid values which were recently determined, when the color tests were made, are twice as high as when the oils were placed in storage. From these data and those reported above it is evident that there is no close correlation between the acidity and color of cod liver oils.

From the foregoing results it appears that the proposed U. S. P. Standard No. 1 (color No. 5 of the scale used in this study) is a too rigorous specification for the maximum color of medicinal cod liver oil. On the other hand the proposed U. S. P. Standard No. 2 (color No. 9 of the scale used in this study) is an altogether too lenient specification. The adoption of the proposed U. S. P. Standard No. 2 would permit the marketing, for medicinal purposes, of cod liver oils which are decidedly darker than the majority of present-day medicinal oils. Hence the results obtained with the oils discussed above indicate that the official color standard for medicinal cod liver oil should be somewhat darker than the proposed U. S. P. Standard No. 1 but decidedly lighter than the suggested U. S. P. Standard No. 2.

SUMMARY.

The color of a series of over one hundred cod liver oils has been studied with apparatus designed for this purpose.

The permanency of the color of solutions of cobalt and iron chlorides suggested as U. S. P. color standards for cod liver oil has been determined and found satisfactory.

In a series of 54 crude cod liver oils the greater portion were found to be darker than the U. S. P. Standard No. 1. However, when some of these oils were chilled and pressed in the usual manufacture of medicinal oil the crude oils became more than enough lighter in color to comply with this standard.

Nearly all the American medicinal oils met the proposed U. S. P. Color Standard No. 1 but about one-third of the European oils were unsatisfactory as regards color. Old oils which had been stored under unfavorable conditions were nearly as light colored as fresh oils of similar origin. However, the acid content of the old oils greatly exceeded that of the fresh oils.

While there is a tendency for dark colored oils to have high acid values and light colored oils to have low acid values there was no consistent correlation between acid value and color of the oils studied.

The proposed U. S. P. standards for color represent a definite advance in the standardization of the color of medicinal cod liver oil. However, the results obtained in this investigation indicate that the official color standard for medicinal cod liver oil should be darker than the proposed U. S. P. Standard No. 1 but decidedly lighter colored than the suggested U. S. P. Standard No. 2.

REFERENCES.

(1) Pharmacopœia of the United States, Tenth Decennial Revision (1926), 263. J. B. Lippincott Company, Philadelphia, Pa.

(2) A. Taub, JOUR. A. PH. A., 22 (1933), 194.

(3) Arny and Taub, JOUR. A. PH. A., 12 (1933), 839.

(4) Data supplied by Mr. Fred Tripp, Mt. Hope Finishing Co., North Dighton, Mass.